CHARACTERIZATION OF 5-HYDROXYTRYPTAMINE-EVOKED ISOMETRIC CONTRACTILE RESPONSES IN AORTIC VASCULAR SMOOTH MUSCLE IN THYROPATHOLOGICAL RATS.

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by
Nicholas P. Edgington
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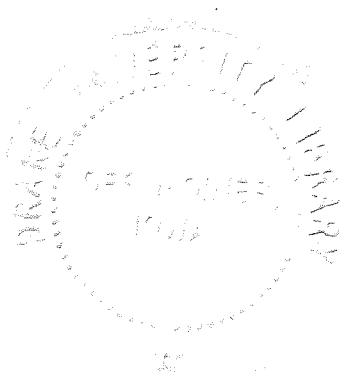

CHARACTERIZATION OF 5-HYDROXYTRYPTAMINE-EVOKED ISOMETRIC CONTRACTILE RESPONSES IN AORTIC VASCULAR SMOOTH MUSCLE IN THYROPATHOLOGICAL RATS

An abstract of a Thesis by
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January 1992
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The problem. In the past, research on the sequellae of thyropathology has utilized a variety of animal models. Recently, vascular smooth muscle has increasingly been used as an effective bioassay to assess physiological function of hormones and ligands. While general effects of thyroid status are well documented, specific thyropathologic-induced changes in 5-hydroxytryptamine (serotonergic;5-HT) receptor function mediating contraction/dilation in rat aorta have not been completely characterized.

Procedure. Aortic rings from hyperthyroid (TRX), hypothyroid (PTU), and euthyroid control (CON) rats were mounted in climate controlled tissue baths and subjected to isometric contraction experiments. The endothelium was removed from some rings (denuded) while others were left intact. All rings were initially contracted with a single dose of 55 mM KCl and then relaxed to normal potassium while concomitantly measuring the time course of this relaxation. All rings were subsequently subjected to dose/response experiments with 5-HT, either in the presence or absence of ketanserin(5-HT2 antagonist) and ICS 205-930(5-HT3 antagonist).

Findings. 5-HT generated a nonsignificant trend toward increased contractile tension in TRX rats, but a significant reduction in contractile tension in rings from PTU rats. These differences were eliminated in the denuded preparations with PTU and control tensions increasing back to TRX levels. There were no differences in sensitivity to 5-HT in the intact preparations in the three thyroid groups, but an increased sensitivity was observed in the TRX denuded preparation. Ketanserin was found to completely antagonize the 5-HT2 receptor-mediated contraction. High concentrations of 5-HT in the presence of high concentrations of ICS 205-930, generated a significantly attenuated contractile tension in rings from TRX rats.

Conclusions. The elimination of a significant difference in the PTU and CON treatment groups in the denuded tissue as compared to the intact tissue, suggests an enhanced 5-HT-mediated release of endothelial derived relaxing factor(EDRF) in PTU rats and reduced release in TRX rats. An increase in sensitivity in the denuded tissues, but not in the intact tissues, suggests an enhanced responsiveness of a 5-HT receptor subtype subserving contraction. The significant effect of micromolar concentrations of ICS 205-930 suggests that an increased contractile response in the TRX group, in light of the apparent lack of 5-HT3 sites in this tissue, may be mediated, in part, by a novel serotonergic receptor mediating contraction. Alternatively, this effect may reflect activity of the ligand at 5-HT1 or 5-HT2 receptors differentially sensitized by thyropathology. These data suggest that thyropathological alterations in 5-HT receptor subtype-mediated responses occur in rat thoracic aorta.

TO NANCY

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INTRODUCTION

Vascular smooth muscle, subserving vasodilation or vasoconstriction, plays a vital role in homeostasis. The homeostasis of the aorta is modulated by a number of complex interacting factors. Hormonal and autonomic signals, vasoactive cellular and neural substances released into the serum, and events at the cellular level regulate the overall tension of the thoracic aorta. Clearly, an imbalance in the maintenance of the tension in the aorta could result in severe pathophysiological consequences.

In the thyropathological state, delicate hormonal feedback mechanisms are disturbed. An excess of thyroxine (T4), is responsible for tachycardia, increased cardiac output, increased glycogen and lipid mobilization, enhanced thermogenesis, tremor, hyperkinetic behavior and sweating(Harrison 1964, Waldstein 1966, Bilezikian and Loeb 1983). It has long been known that many of these clinical features resemble the effects of increased sympathetic activity (Coville and Telford 1970). Previous studies have attempted to elucidate such thyroid-sympathetic-adrenal medullary interrelationships.

Early radioligand binding studies of turkey erythrocyte ß-adrenergic receptors have suggested that hyperthyroidism did not change the number(B_{max}) of ß-adrenergic receptors, or the activity of ß-adrenoreceptor linked-adenylate cyclase

(Bilezikian, Loeb, and Gammon 1979). However, levels of cyclic AMP were increased.

Conversely, in the hypothyroid state, ß-adrenergic receptor B_{max} and the level of adrenergic-responsive cyclic AMP were found to be decreased (Bilezikian, Loeb, and Gammon 1979).

In hyperthyroid rat heart membranes, radioligand binding studies employing selective adrenergic antagonists demonstrated increased ß-adrenergic receptor B_{max}, with no differences in ß-adrenergic receptor affinity(K_D) for specific agonists or antagonists (Williams et al. 1977, Ciaraldi and Marinetti 1977, Tsai and Chen 1978, Kunos, Mucci, and O'Regan 1980). Thyroxine treatment potentiated positive inotropic response mediated by the ß-adrenergic receptors in guinea pig atria and rabbit papillary muscles (Hashimoto and Nakashima 1978). A decreased ß-adrenergic receptor B_{max} in rat heart was found in the absence of thyroid hormone(Banerjee and Kung 1977, Ciaraldi and Marinetti 1977, Kunos, Mucci, and O'Regan 1980). These studies have demonstrated that in cardiac tissue, changes in thyroid state are accompanied by changes in ß-adrenergic number. These differential responses have not been clearly delineated in aortic tissue.

There is conflicting evidence regarding the effects of thyroid state on the rat aorta. A recent study has found that ß-adrenergic receptor density is increased by approximately 4-fold in the hyperthyroid state, and reduced

by a factor of 0.68 in the hypothyroid state (Gunasekera and Kuriyama 1990).

Discernable decreases in ß-adrenergic receptor density in other tissues such as adipocytes (Ciaraldi and Marinetti 1978), reticulocytes (Malbon 1980, Stiles et al. 1981), and cerebral cortex (Gross, Brodde, and Schumann 1980), have also been reported in hypothyroid animals. In most tissues studied, changes in ß-adrenergic receptor number are seen without concurrent changes in receptor affinity. These studies have elucidated the plasticity of ß-adrenergic receptor density induced by thyropathology.

The α-adrenergic receptor response has not been as completely characterized as the ß-adrenergic receptor response. In general, thyroid status has been shown to regulate α-adrenergic receptor density and responsiveness. Hyperthyroid hearts have a 40% reduction in the number of α-adrenergic receptors with a simultaneous decrease in receptor affinity by 2 to 4 fold (Ciaraldi and Marinetti 1977, Williams and Lefkowitz 1979, Chang and Kunos 1981, Bilezikian and Loeb 1983). Although no changes in number or affinity of α-adrenergic receptors were found in hyperthyroid hamster adipose tissue (Garcia-Sáinz et al. 1981), a 70% decrease in number was determined in rat liver, with no associated changes in affinity (Malbon and LoPresti 1981).

A decrease in α-adrenergic receptors in hypothyroid rat heart that was equivalent to the increase seen in hyperthyroid heart tissues has been reported (Ciaraldi and

Marinetti 1977, Ciaraldi and Marinetti 1978). Hamster adipose tissue in the absence of thyroid hormone has shown no differences in number or affinity for the α-adrenergic receptor (Giudicelli, Lacasa, and Agli 1980). In contrast, the hypothyroid rat liver exhibits a significant reduction in the number of α-adrenergic receptors without concurrent changes in affinity (Noguchi 1983). Recently, Gunasekera and Kuriyama(1990) have shown that α-adrenergic receptor density was increased in the hyperthyroid state, and decreased in the hypothyroid state.

This research on the relationship between thyroid status and adrenergic responsiveness has shown that both the α- and ß-adrenergic receptor systems, in a variety of tissues, can be regulated by thyroid status.

Although thyroid excess and deficit-associated changes in catecholamine receptor density and sensitivity in rat thoracic aorta have been well documented, studies of the effects of thyroid status on heterogenous populations of 5-hydroxytryptamine(serotonin; 5-HT) receptors in this tissue have not been undertaken. As 5-HT receptors function in concert with adrenergic receptors in the maintenance of vascular tone (Marzini, Maggi, and Meli 1986), it is possible that 5-HT receptor density and sensitivity may also be differentially regulated in an altered thyroid status.

Peripheral 5-HT receptors were initially characterized into two distinct types; "D" and "M", using the non-specific antagonists dibenzyline and morphine, respectively(Gaddum and

Picarelli 1957). As more specific pharmacological antagonists became available, 5-HT_1 and 5-HT_2 sites were distinguished using the antagonists spiperone and D-lysergic acid diethylamide(LSD) in rat brain membranes(Peroutka and Snyder 1979). Bradley et al.(1986) proposed a classification scheme to integrate the two proposed classifications, designating the receptor groups as '5-HT_1-like', 5-HT_2, and 5-HT_3. The 5-HT_1 and 5-HT_2 receptor types have been further subdivided into several distinct subtypes. Table 1 summarizes the current nomenclature and knowledge of 5-HT receptor function in vascular smooth muscle.

The definition of the receptor group categorized as '5-HT_1-like' has been left as broad as possible to allow the further identification and characterization of novel 5-HT_1 receptors, as the number of new selective serotonergic agents become available. There are three criteria that define this group of receptors (Bradley et al. 1986). First, the receptor is potently antagonized by methiothepin and/or methysergide. Although these antagonists are not exclusively selective for 5-HT_1 sites, they show a high affinity for them. Second, 5-HT_1-like receptors are not inhibited by antagonists that are selective for the 5-HT_2 or 5-HT_3 sites, such as ketanserin, ritanserin (5-HT_2),ICS 205-930, MDL 72222 (5-HT_3). Third, this receptor's response to the 5-HT_1 agonist 5-carboxamidotryptamine(5-CT) must be equal to or greater than that of 5-HT (Bradley et al. 1986). Although the 5-HT receptor on the pig coronary artery

endothelium mediating relaxation shows a strong similarity to the 5-HT_{1D} receptor subtype(Schoeffter and Hoyer 1990), it would be premature to assign a 5-HT1 receptor subtype on the rat thoracic aorta endothelium 5-HT receptor.

The mechanisms of signal transduction initiated by the ligand-binding to the '5-HT1-like' endothelial receptor, and completed by the contraction or dilation of the aortic vascular smooth muscle have not been fully elucidated. Efforts to directly link 5-HT-induced release of a diffusible vasodilatory substance into vascular smooth muscle is still lacking. However, cascade-superfusion experiments have demonstrated the 5-HT-mediated release of an endogenous vasodilatory ligand from endothelial cells of a donor aortic ring can relax an acceptor aortic ring which immediately follows that of the donor ring (Cohen 1989). Previous research has shown that upon removal of the endothelium, 5-HT- and acetylcholine(ACh)-mediated endothelium-dependent dilation of vascular smooth muscle is abolished (Cohen and Vanhoutte 1986, Furchgott and Zawadski 1980). Endothelial-derived relaxing factor (EDRF) is a short-lived soluble agent released by the aortic endothelium in response to a variety of substances including 5-HT and ACh, and mediates relaxation in the rat thoracic aorta (Furchgott 1983). EDRF acts in a manner similar to nitrate vasodilators, and shows certain similarities to nitric oxide to induce an increase in cellular levels of cyclic guanosine monophosphate (cGMP)(Rapaport and Murad 1983).

The role of endothelial-derived contracting factor(EDCF) has been characterized in more detail. This factor is released from the endothelium, presumably in response to the binding of plasma-born vasoconstrictors on the intimal side of the aortic endothelium. Recently, a 21-amino acid peptide called endothelin has been isolated from porcine endothelial cells in culture (Yanagisawa et al. 1988). Through sequence analysis, the porcine and human endothelin peptides have been found to be identical, and have been termed endothelin-1 (ET-1). The endothelin-2 (ET-2) differs by two amino acid substitutions from ET-1. The isoform isolated from rat shares certain homologies with ET-1 and ET-2, and has been termed endothelin-3 (ET-3)(Inoue et al. 1989). Data indicate that EDCF and endothelin share many similarities, and may in fact be the same molecule (Rapoport and Highsmith 1990). Upon release, endothelin binds to a transmembrane endothelin receptor on the membrane of the vascular smooth muscle. The signal is transduced via a pertussis toxin-insensitive G protein, which is coupled to phospholipase-C (Takuwa et al. 1990). Endothelin has been shown to induce contraction through several related pathways; Phospholipase-C hydrolyzes three phosphatidylinositides to form diacylglycerol(DAG) and inositol triphosphate(IP3) (Rapoport and Highsmith 1990, Sugiura et al. 1989). These two products function as intracellular second messengers. IP3 mediates the release of calcium stores from the endoplasmic reticulum, which allow the vascular smooth muscle to contract. The main source of

calcium, mobilized by endothelin to elicit contraction, enters through voltage-independent calcium channels on the vascular smooth muscle membrane (Huang, Hisayama, and Takayanagi 1990). The activation of DAG and phospholipase A2 both lead to the release of arachidonic acid. The metabolism of arachidonic acid by cyclooxygenase and lipoxygenase enzymes forms many vasoactive substances such as thromboxanes, prostaglandins, and leukotrines which may play an essential role in sustaining endothelin-mediated contraction (Reynolds and Mok 1990). The distribution and vascular actions of the heterogenous 5-HT receptors, EDRF, and EDCF are shown in Figure 1.

This thesis will deal with the characterization of 5-HT-evoked isometric contractile responses in aortic vascular smooth muscle in thyropathologic rats. A central hypothesis of this thesis is that the modulation of adrenergic responses in thyropathologic rat aorta may be accompanied by similar alterations in indolalkylaminergic responses. Given that the α-adrenergic receptor and the 5-HT_2 receptor both mediate contraction of vascular smooth muscle utilizing similar signal trandsduction cascades (Van Zwieten and Timmermans 1987, Roth et al. 1984), it is tenable to hypothesize an excess or deficit of thyroid hormone may induce parallel changes in contractile responses. Conversely, thyropathology may mediate opposite effects in the two populations of receptors, implicating post-receptor modifications in mechanisms of vascular smooth muscle contraction. In order

to elucidate thyropathologic changes of 5-HT-induced contractility of rat aorta, this study used mechanical denudation (removal) of the aortic endothelium to differentiate between 5-HT-mediated responses that occur on the endothelium, to those which occur on the vascular smooth muscle. In addition, the selective 5-HT_2 antagonist ketanserin, and 5-HT_3 antagonist ICS 205-930, were perfused through endothelial-intact aortic rings of the three thyroid states, to differentiate between 5-HT-mediated responses mediated by specific 5-HT-receptor types. An additional purpose of this thesis was to examine the effects of thyropathology on vascular smooth muscle repolarization. As an indication of rate of repolarization of the vascular smooth muscle, the rate of relaxation was measured after a KCl-mediated contraction. Several mechanisms have been proposed for the KCl-mediated contraction of vascular smooth muscle. KCl-induced alterations of the membrane potential may be mediated by changes in the membrane permeability to sodium or potassium ions, the electrogenic sodium-potassium transport system, and/or the influence of the intracellular sodium concentration on calcium transport (Sparks 1980). This measure may indicate thyropathologic-induced changes in cell membrane ion channels involved in the repolarization of the vascular smooth muscle.

Table 1. 5-Hydroxytryptamine receptors relevant to the maintenance of vascular smooth muscle tone.

Receptor Subtype	Tissue Localization	Transduction Pathway
5-HT2	vascular smooth muscle endothelial cells platelets	stimulation of phospholipase C accumulation of inositol phosphates stimulation of protein kinase C coupled to a G-protein; mediates contraction
5-HT1B[a]	WKR rat aortic smooth muscle cell culture	coupled to a G-protein negatively linked to adenylate cyclase
'5-HT1-like'	endothelium of thoracic aorta	transduction pathway is not known; may mediate contraction and/or dilation
5-HT1D[b]	endothelium of pig coronary arteries	

[a] Jazayeri, Meyer and Kent (1989).

[b] Schoeffter, Waeber, Palcios and Hoyer (1990).

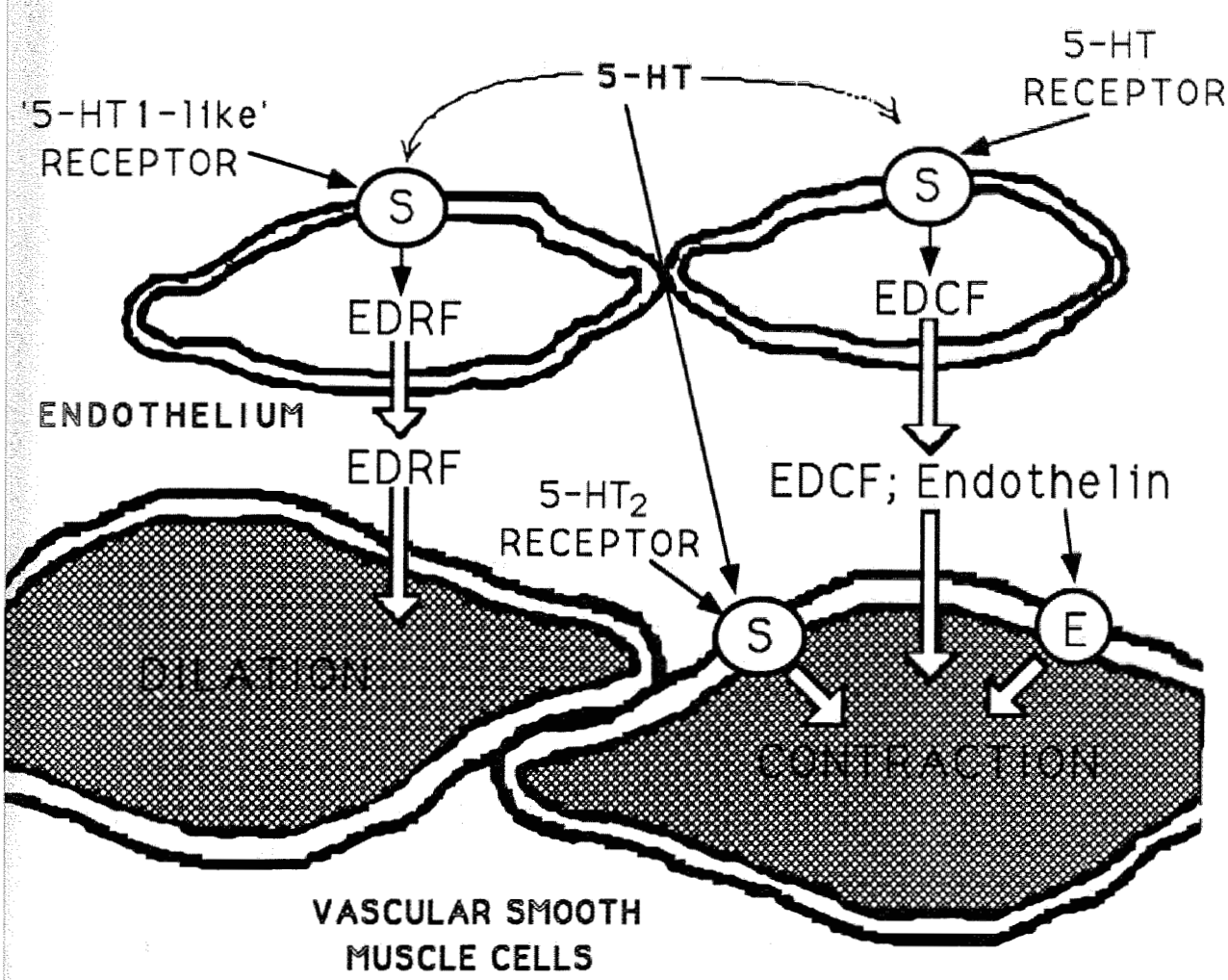


Figure 1. Functional relationship between endothelium and vascular smooth muscle in response to 5-HT.

MATERIALS AND METHODS

Experimental animals. The subjects were male Sprague-Dawley rats weighing between 300 and 400 grams. A total of 36 rats were used, with 12 in each treatment group. The rats were housed 2 per cage. One group was rendered hyperthyroid by daily intraperitoneal injection of 200 µg of l-thyroxine(TRX) in a 0.2 ml volume of physiological saline. The second group was rendered hypothyroid by the administration 0.1% 2-thiouracil (PTU) in the drinking water. A third euthyroid control(CON) group was maintained. The three groups were allowed Purina rat chow and water *ad libitum* for the duration of the experiment. The animal care unit was maintained at 25°C, and light/dark cycles were alternated every 12 hours.

Dissection and Mounting of the Aortic Ring. Following three weeks of treatment, rats were sacrificed by cervical dislocation. The thoracic aorta was quickly and carefully removed and dissected free of connective tissue in oxygenated buffered physiological saline solution(PSS), pH adjusted to 7.4, and maintained at 37°C in a preparation dish. The composition of the PSS was as follows (mM): NaCl, 115; KCl, 5; $CaCl_2$, 2.5; $MgCl_2$, 1.2; EDTA, 0.026; NaH_2PO4, 1.2; $NaHCO_3$, 25. The aorta from each rat was cut into eight rings, each approximately 2 mm in width, and were either left intact, or denuded by removal of the endothelial cell layer. The

denudation of the rings was done by inserting a pair of angled forceps into the lumen of the vessel and gently rolling the ring approximately four times on a piece of filter paper at the bottom of the preparation dish. The rings were then mounted between two wire hooks in a 6ml tissue bath (Radnotti) containing PSS (Figure 2) . The tissue bath was maintained at 37°C and continually gassed with 95% O_2 / 5% CO_2. Changes in tissue isometric force were measured with strain gauge transducers and recorded on a Beckman RB, and a Sensormedic R511 Dynograph.

Data Collection After the dynograph was calibrated, rings were preloaded to one gram of passive tension. The rings were allowed to incubate for two hours(Figure 3) . During this time, tissue bath PSS was replaced every fifteen minutes. After two hours of incubation, the rings were challenged with a 55mM KCl solution to determine viability. After a maximum contraction was achieved, the KCl solution was washed, allowing the rings to relax. The time course of relaxation of the KCl-induced contraction was measured to determine if a differential repolarization rate occurred in the three thyroid states, and in intact and denuded tissues. To insure that the endothelium was actually removed by this procedure, pilot studies were conducted in which separate rings were precontracted with norepinephrine(NE), and then exposed to 1 μM acetylcholine(ACh). An abolishment of relaxation caused by ACh was taken as evidence of successful removal. Once a baseline of 1 gm of tension was reachieved,

either no drugs were added, or the 5-HT_2 antagonist, ketanserin, or 5-HT_3 antagonist ICS 205-930, was added to the tissue bath solution. The 5-HT antagonists were allowed to bind for fifteen minutes. After this time, cummulative concentrations of serotonin(5-HT) were added, in a range of $1x10^{-10}$ to $1x10^{-5}$ m/L. After a maximal response to the highest dose of 5-HT was achieved, rings were removed from the apparatus, blotted on filter paper, and weighed on a Mettler balance to determine the total wet weight.

Drugs Drugs used were serotonin(5-HT) (Sigma Chemical Co., St. Louis, MO); 5-HT_2 antagonist, ketanserin (Research Biochemicals Inc., Natick, MA); 5-HT_3 antagonist, ICS 205-930 (Research Biochemicals Inc.); l-thyroxine(TRX) (Sigma Chem. Co.); and 2-thiouracil(PTU) (Sigma Chem. Co.).

Data Analysis The contractile force of each ring was measured in milligrams(mg). The contractile force was normalized to the wet weight of the ring as mgs of tension/mgs of wet ring weight. The chart recorders were calibrated so that 1 mm of pen deflection reflected 100 mg of generated tension. The percent of the maximum contraction for each incremental dose of 5-HT was calculated using the following formula:

$$\% \text{ MAX} = \frac{\text{Response[5-HT]}}{\text{Max. Response of ring}} \times 100$$

The percent relaxation, from the wash off of the KCl-induced contraction, was calculated using the preceding method. The

normalized tension generated and the percent maximum contraction of the rings in each group were averaged and standard errors (S.E.) were calculated. The Students t-test (two-tailed) for independent samples was used to compare the means of the TRX and PTU treatment groups to the control group, with a value of $p \leq 0.05$ considered significant. The dose at which a half-maximal response was achieved (ED_{50}) was determined by the SSPX program logit/probit.

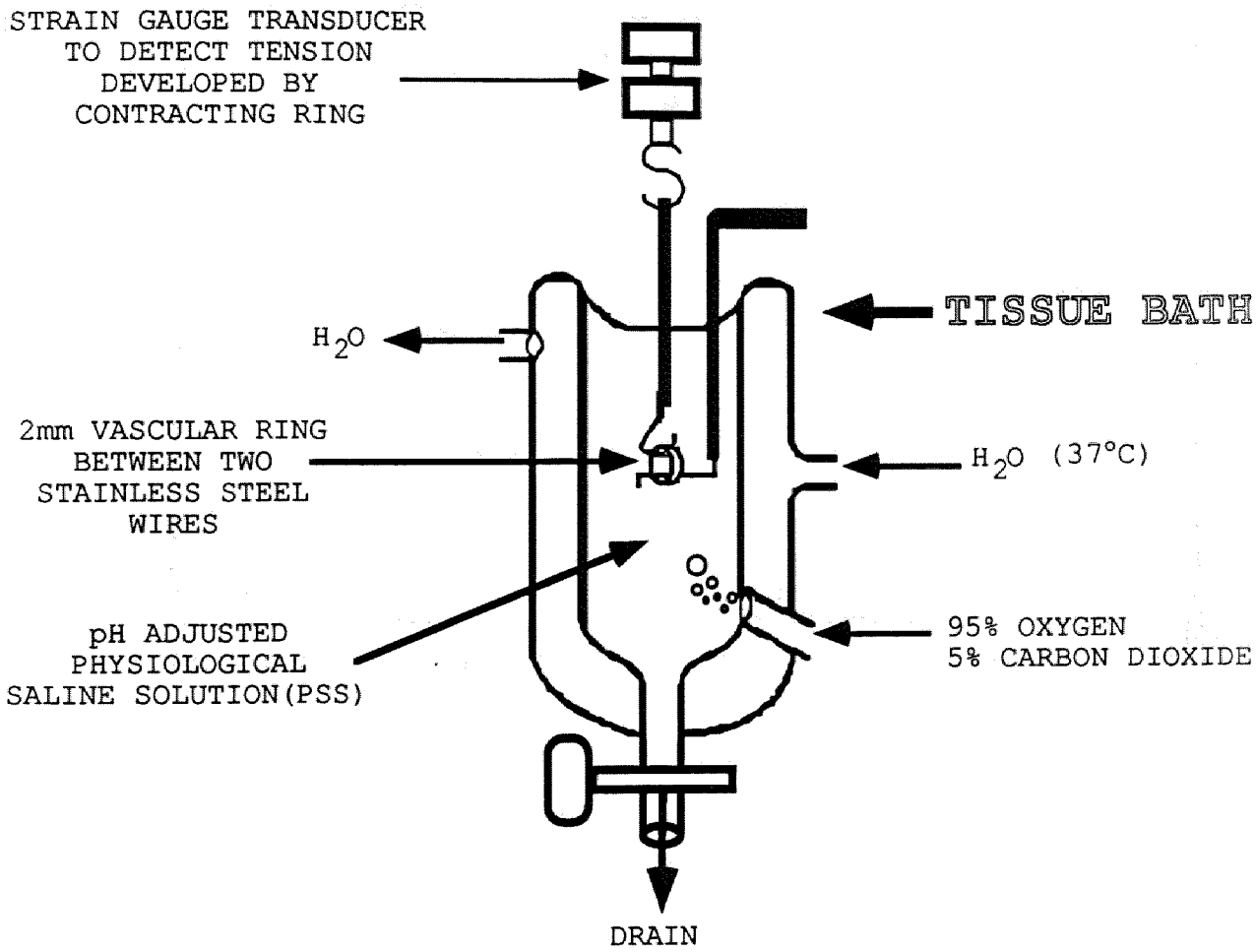


Figure 2. Tissue bath apparatus.

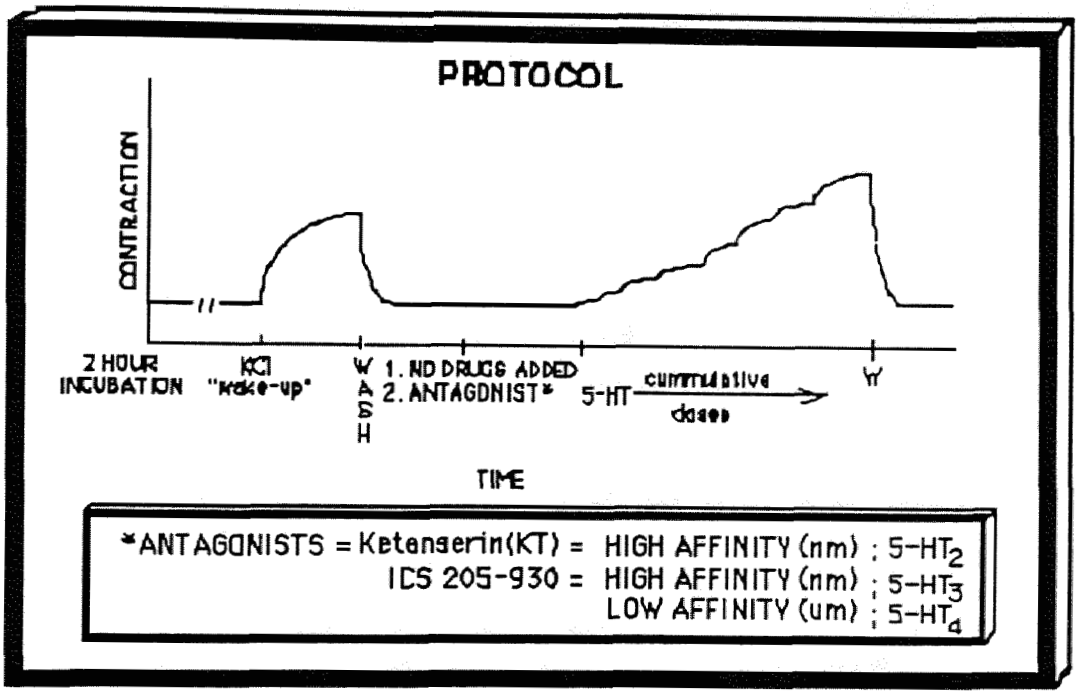


Figure 3. Protocol and drugs used.

RESULTS

Time Course of Relaxation after Potassium-induced Contraction

To determine viability of the tissue, the aortic rings were contracted with a 55mM potassium-chloride solution. After a maximal response was attained, the potassium solution was washed off, and the rate of relaxation was measured for twenty minutes.

The data compiled from the relaxation time were plotted as the percent relaxation. The percent relaxation is an indication of the rate of repolarization of the vascular smooth muscle. The intact tissues of the two thyropathological states showed significant differences when compared to the control in time that was required to achieve 100 percent relaxation. The hypothyroid rings (PTU-treated rats), which achieved the fastest relaxations, were completely relaxed after approximately six minutes. The control and hyperthyroid rings achieved complete relaxation after ten and twenty minutes respectively (Figure 4a). Differences in the denuded (endothelium-removed) preparations of the two thyropathological states were also significant when compared to the denuded control (Figure 4b). The hyperthyroid(TRX) aortic rings were not able to achieve the previous preloading tension after twenty minutes (Figure 4b).

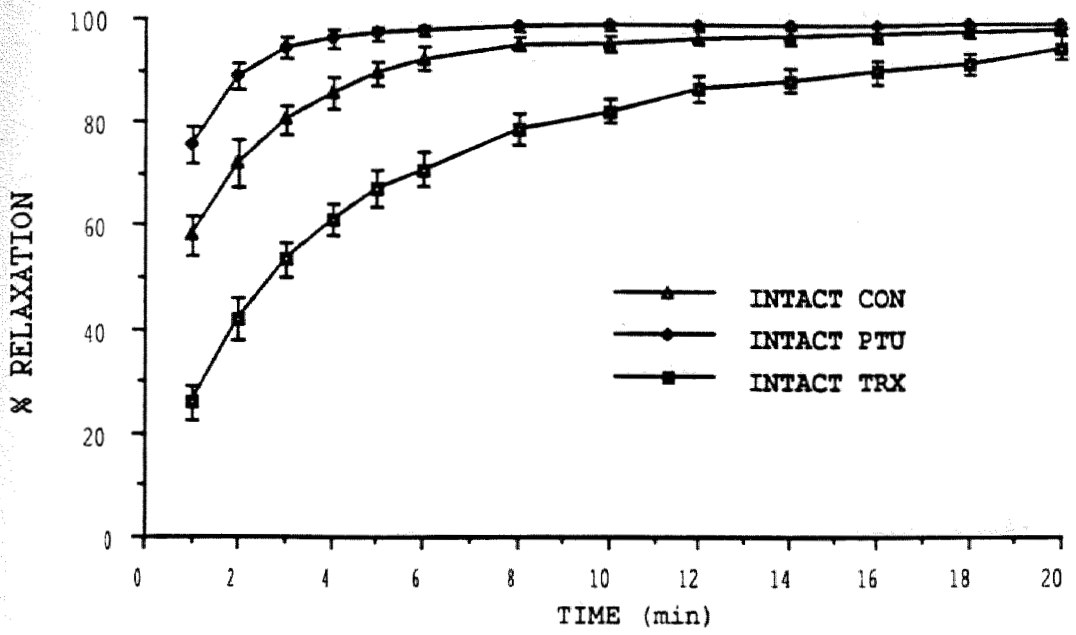


FIGURE 4a. Time course of percent relaxation after a maximal KCl-induced contraction in the intact thyroid states.

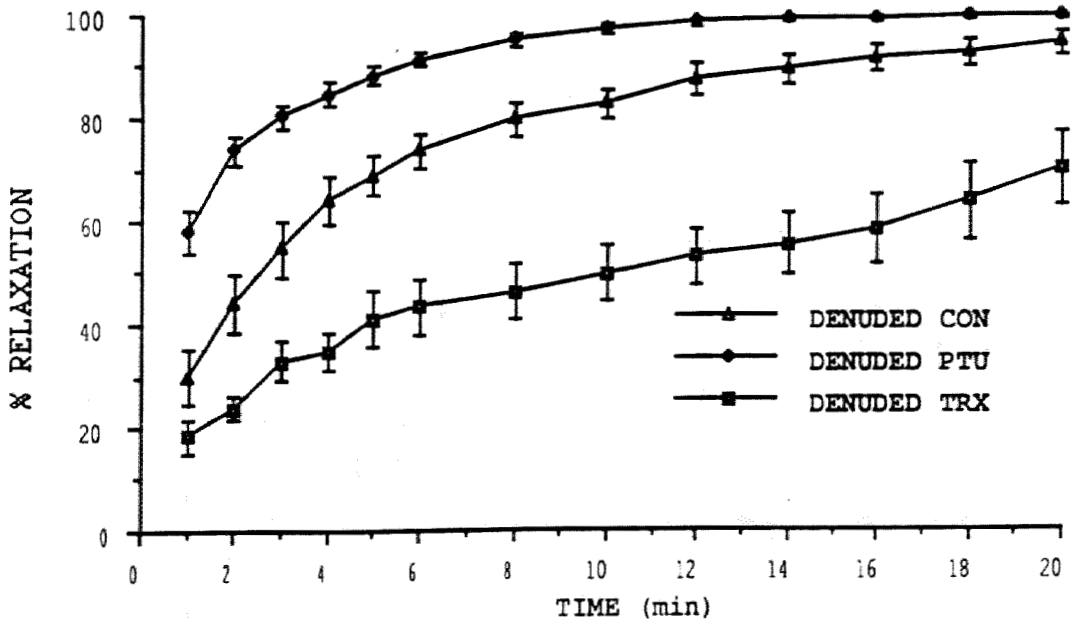
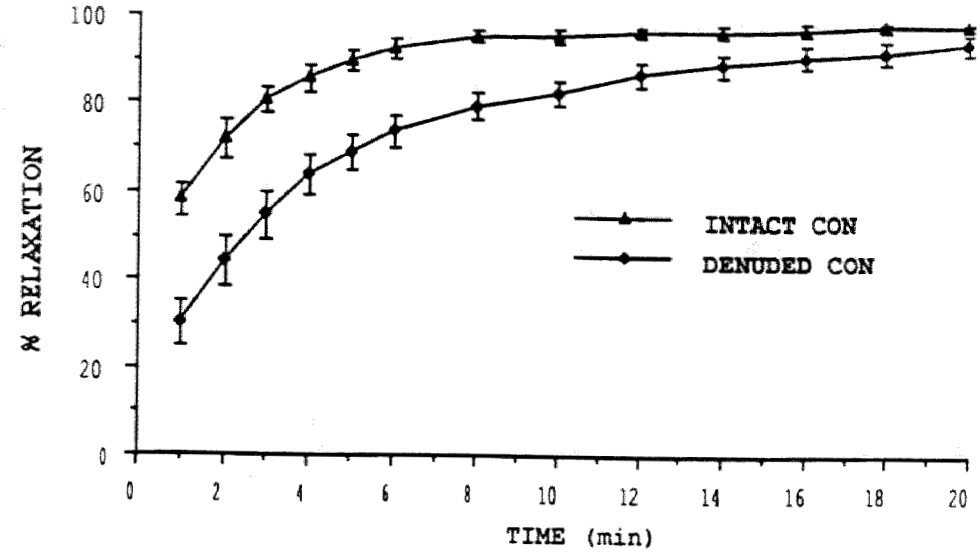
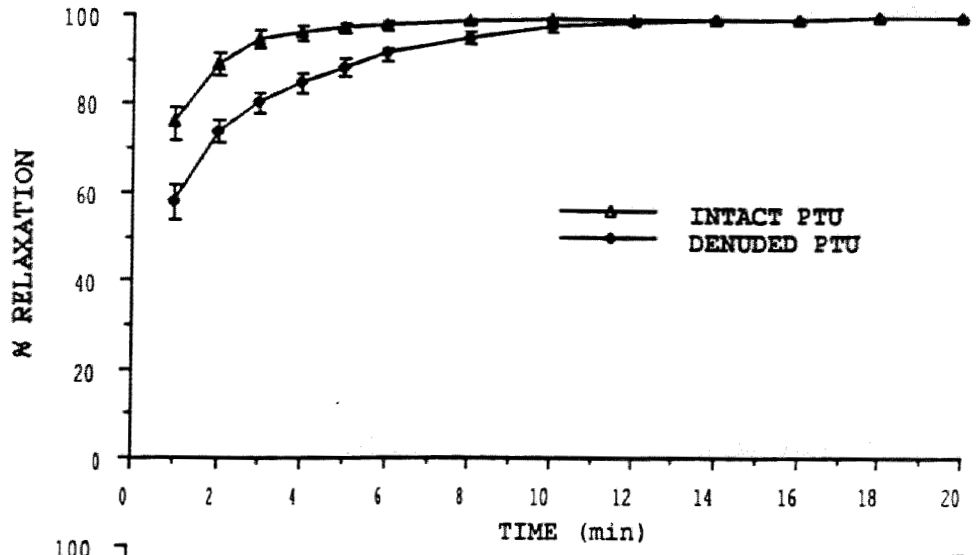
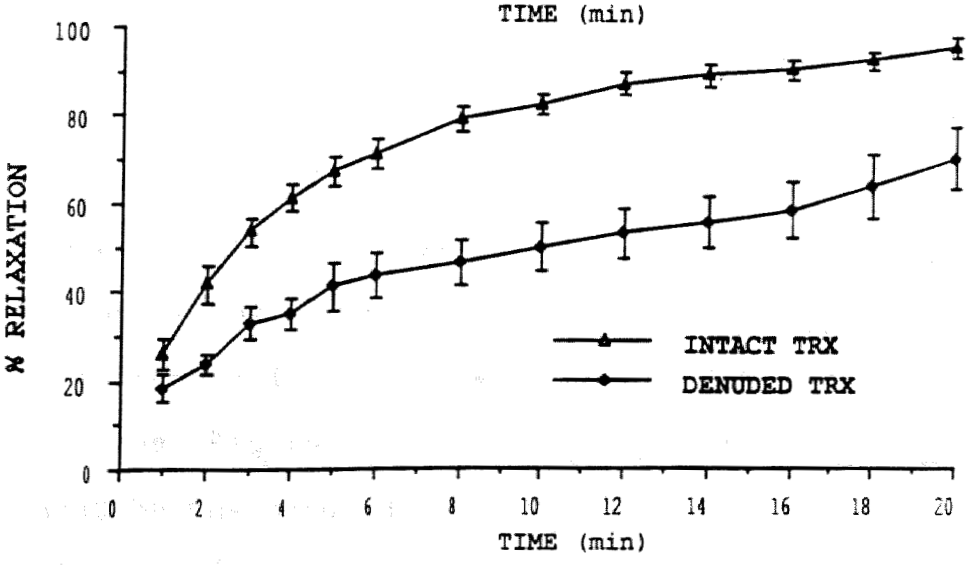


FIGURE 4b. Time course of percent relaxation after a maximal KCl-induced contraction in the denuded thyroid states.







FIGURES 4c, 4d, 4e. Time course of percent relaxation after a maximal KCl-contraction of intact and denuded tissues in the thyroid states. All data points from the denuded thyroid states are significantly less when compared to the intact thyroid states.

The overall time course of relaxation of the denuded tissues in the three thyroid groups were significantly attenuated ($p \leq 0.05$) when compared to their intact counterparts (Figures 4c, 4d, 4e).

Tension Generated in Response to 5-HT in Aortic Rings From the Three Thyroid Groups

The data of the increasing doses of 5-HT were plotted as the amount of raw tension generated(mg of tension/mg of ring weight), and the percent maximum response.

A comparison of the three thyroid states in intact tissues showed a general trend towards an increased tension generated in the hyperthyroid tissue, and diminished tension generated in the hypothyroid tissue when compared to the control. Although the hyperthyroid tissues response was nonsignificantly different than control tissues response, the hypothyroid tissues were significantly different at the highest two doses of 5-HT ($p \leq 0.05$) (Figure 5a). In the denuded preparations, in which the endothelial cell layer had been removed, the control and hyperthyroid rings demonstrated slight increases in tension when compared to their respective intact rings (Figure 5b). However, the maximal tension generated by the denuded hypothyroid rings increased approximately 200 mg/mg, when compared to the intact hypothyroid rings response, to equal the maximal tension achieved by the denuded hyperthyroid and control rings

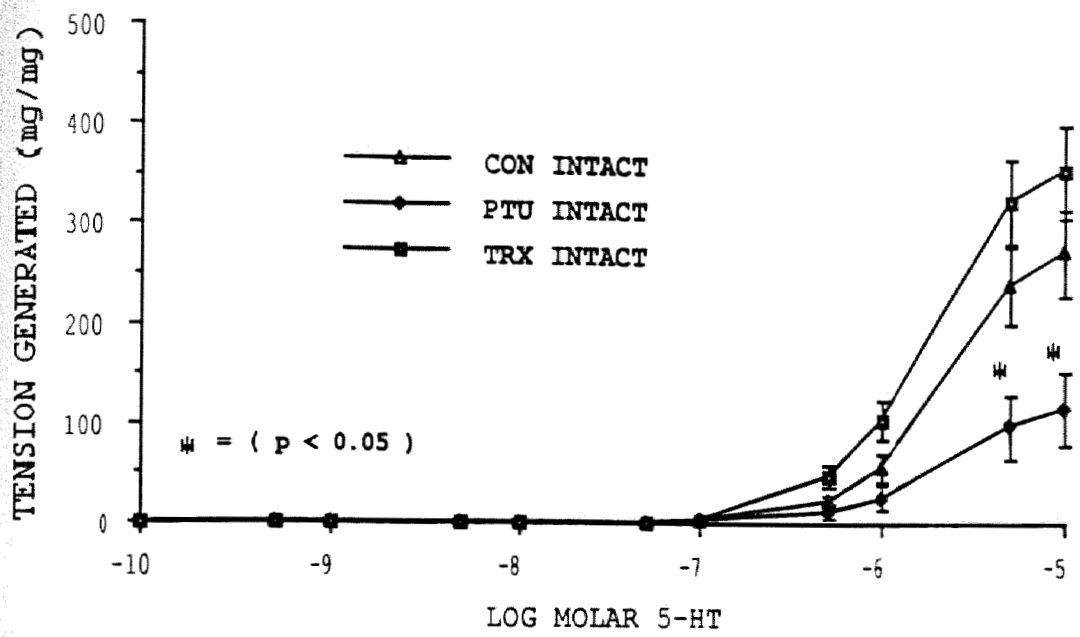


FIGURE 5a. Effects of cumulative concentrations of 5-HT on aortic rings from (CON)euthyroid, (PTU)hypothyroid, and (TRX) hyperthyroid rats. Generated tension is expressed as mg/mg of wet ring weight. Each point represents the mean tension generated for 10 determinations(+/- SEM).

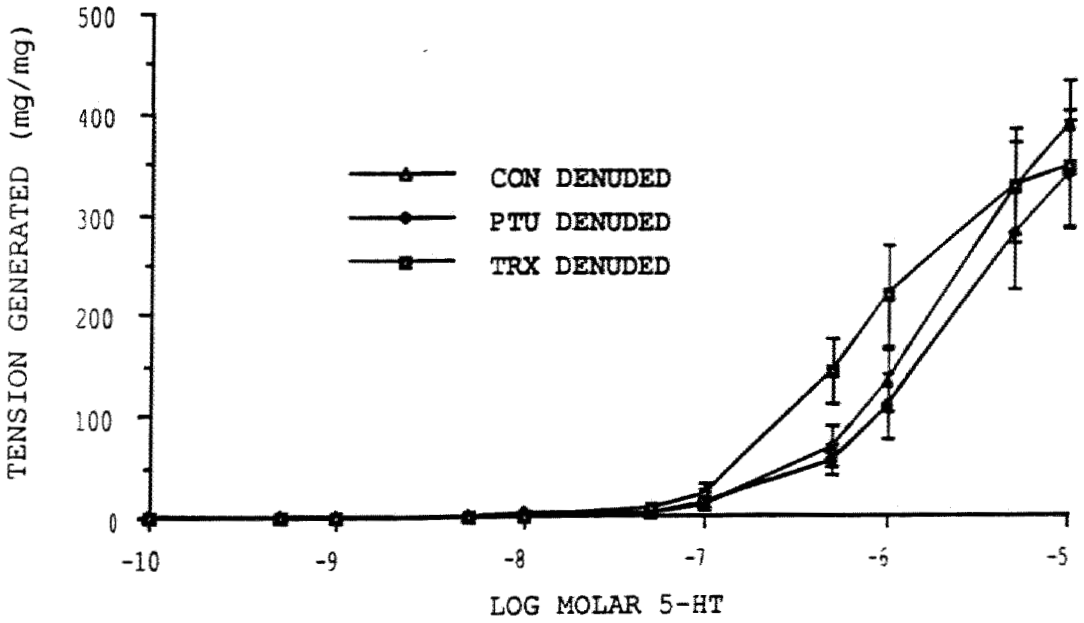


FIGURE 5b. Effects of cumulative concentrations of 5-HT on denuded(endothelium-removed) aortic rings from euthyroid, hypothyroid, and hyperthyroid rats.

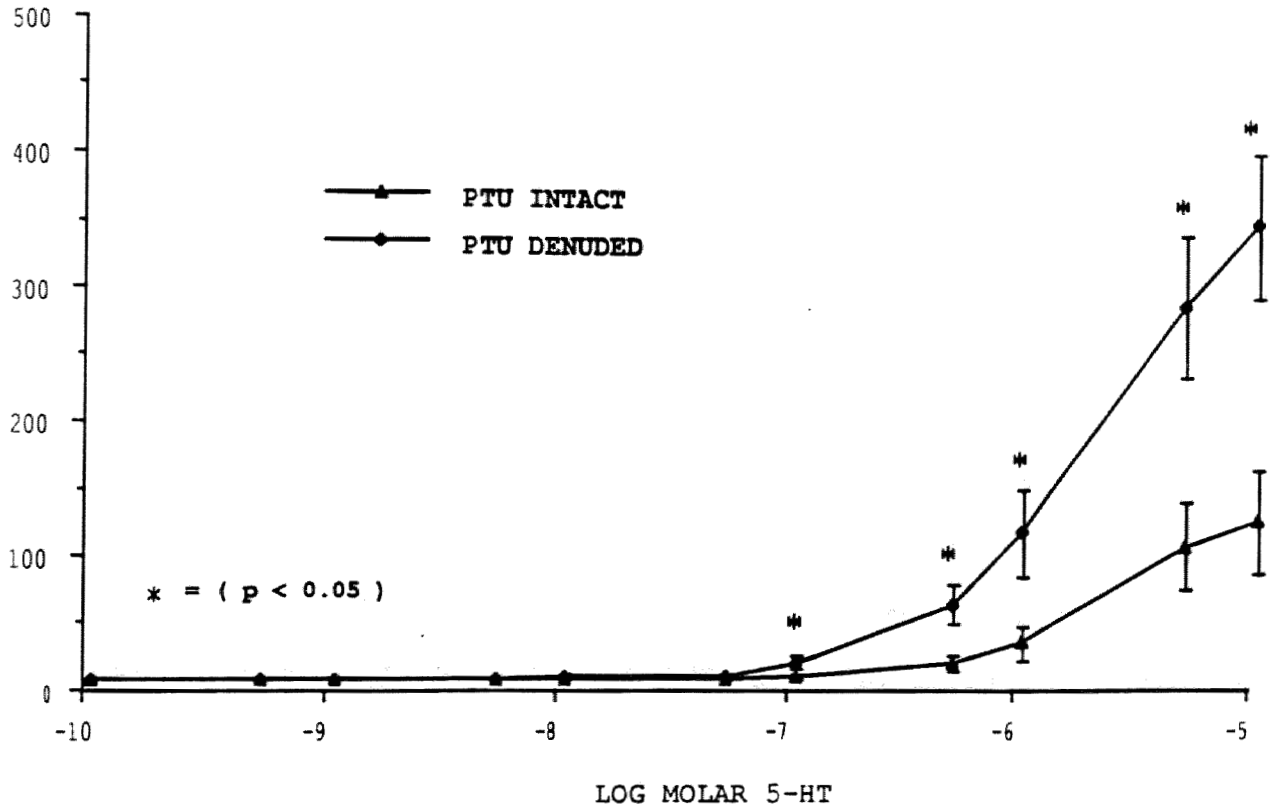


FIGURE 5c. Effects of cumulative concentrations of 5-HT on intact and denuded aortic rings from hypothyroid rats.

(Figure 5b). Although a general trend toward increased tension generated in denuded preparations when compared to intact preparations was observed, the denuded hypothyroid rings demonstrated the largest increase in tension, when compared to the intact hypothyroid tissue (Figure 5c).

Percent Maximum Response to 5-HT in Aortic Rings from the Three Thyroid Groups

The percent maximum contraction is a calculation which gives an indication of the sensitivity of the receptor for the ligand. The percent maximum contraction of the intact preparations was nonsignificantly different among the three thyroid states (Figure 6a). The percent maximum contraction of the denuded preparations showed no significant difference in sensitivity in the control and hypothyroid tissues (Figure 6b). However, in the denuded hyperthyroid tissues, a significant increase in sensitivity was shown at doses of 0.5 μM - 50 μM of 5-HT when compared to the denuded aortic tissue receiving the control vehicle (Figure 6b).

Tension Generated in Response to 5-HT in Intact and Denuded Aortic Rings

The responses of the intact and denuded aortic rings of the thyropathologic and control rats were compared. The tension generated in the denuded control and hyperthyroid rings when compared to their respective intact rings were

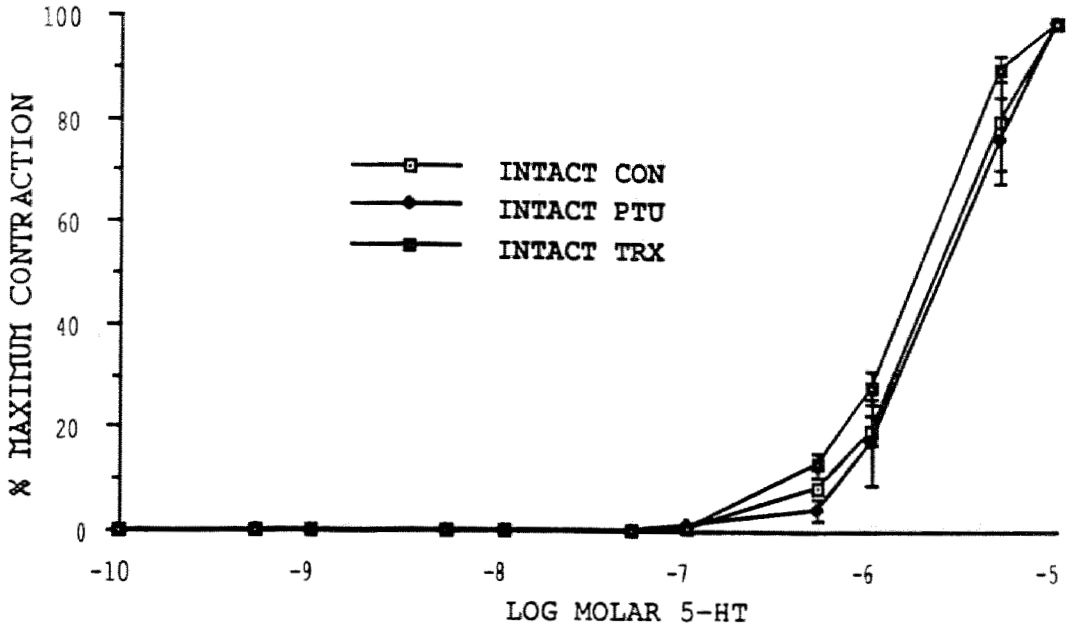


FIGURE 6a. Percent maximum contractions of cumulative concentrations of 5-HT on intact aortic rings from euthyroid, hypothyroid, and hyperthyroid rats.

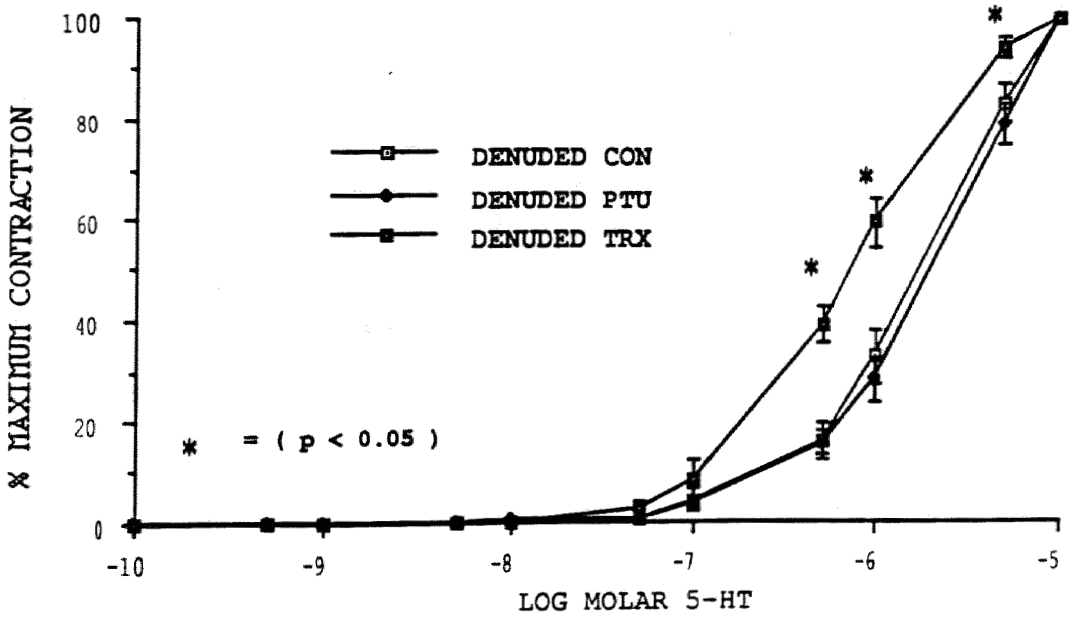


FIGURE 6b. Percent maximum contractions of cumulative concentrations of 5-HT on denuded aortic rings from euthyroid, hypothyroid, and hyperthyroid rats.

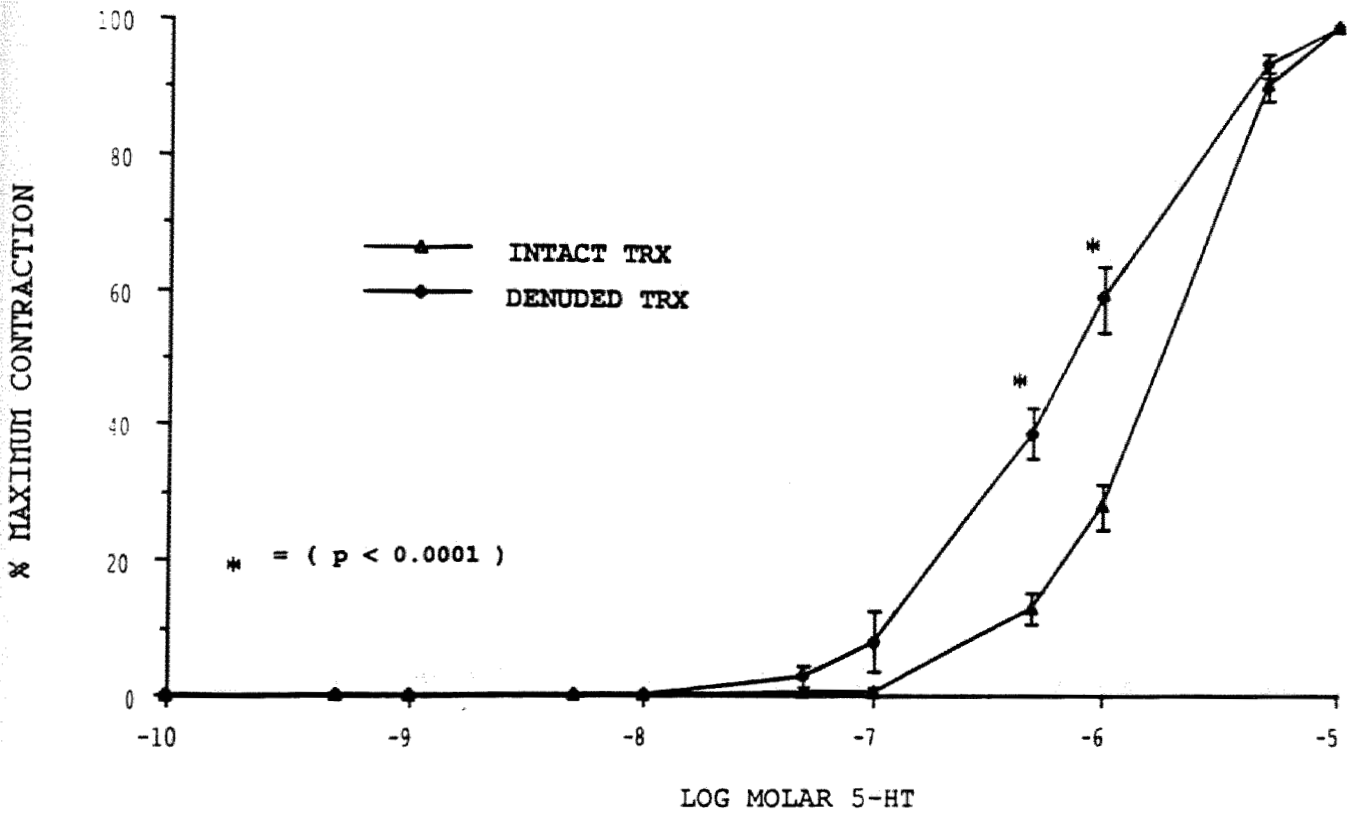


FIGURE 6C. Percent maximum contractions of cumulative concentrations of 5-HT on intact and denuded aortic rings from hyperthyroid rats.

significant at 0.5 μM and 1 μM doses of 5-HT (Figures 5a,5b). The denuded rings of the hypothyroid rats demonstrated a significant increase in the raw tension achieved when compared to the intact hypothyroid ring (Figure 5c).

Percent Maximum Response to 5-HT in Intact and Denuded Aortic Rings

A comparison between percent maximum contractions of the intact and denuded rings revealed a nonsignificant trend toward increased sensitivity in the denuded rings from control and hypothyroid rats (Figures 6a,6b). The denuded hyperthyroid rings showed a significant increase in percent maximum contraction achieved when compared to the intact hyperthyroid rings (Figure 6c).

Tension Generated in Response to 5-HT in Intact Rings Pretreated with ICS 205-930 and Ketanserin

The effects of the 5-HT_2 antagonist ketanserin and 5-HT_3 antagonist ICS 205-930 were investigated in the intact preparations of the three thyroid states..The 5-HT_3 antagonist ICS 205-930 nonsignificantly attenuated 5-HT-mediated contraction at micromolar concentrations in the control and hypothyroid aortic rings when compared to their untreated counterparts (Figures 7a,7b). The tension generated in the ICS 205-930-treated hyperthyroid rings demonstrated a significantly attenuated response at the 5 μM

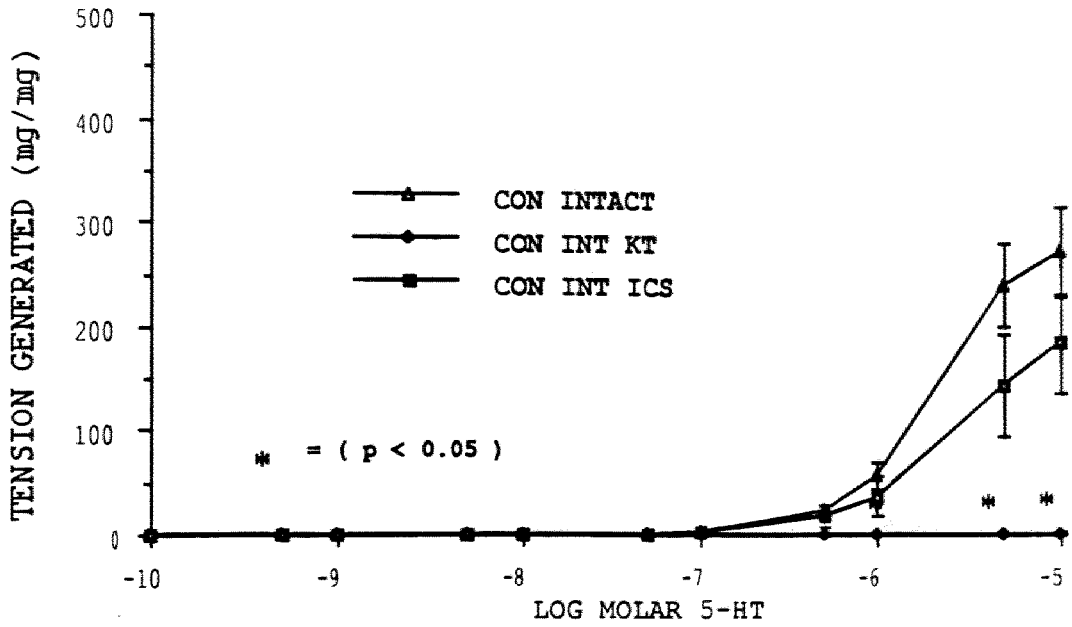


FIGURE 7a. Effects of cumulative doses of 5-HT on intact and ketanserin or ICS 205-930 pretreated aortic rings from euthyroid rats.

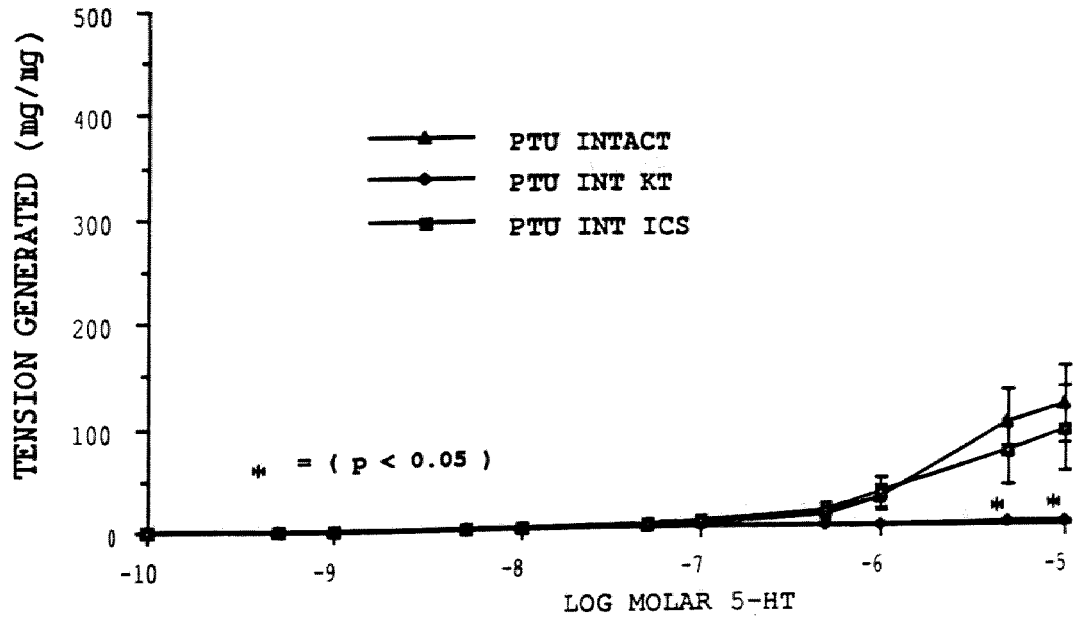


FIGURE 7b. Effects of cumulative doses of 5-HT on intact and ketanserin or ICS 205-930 pretreated aortic rings from hypothyroid rats.

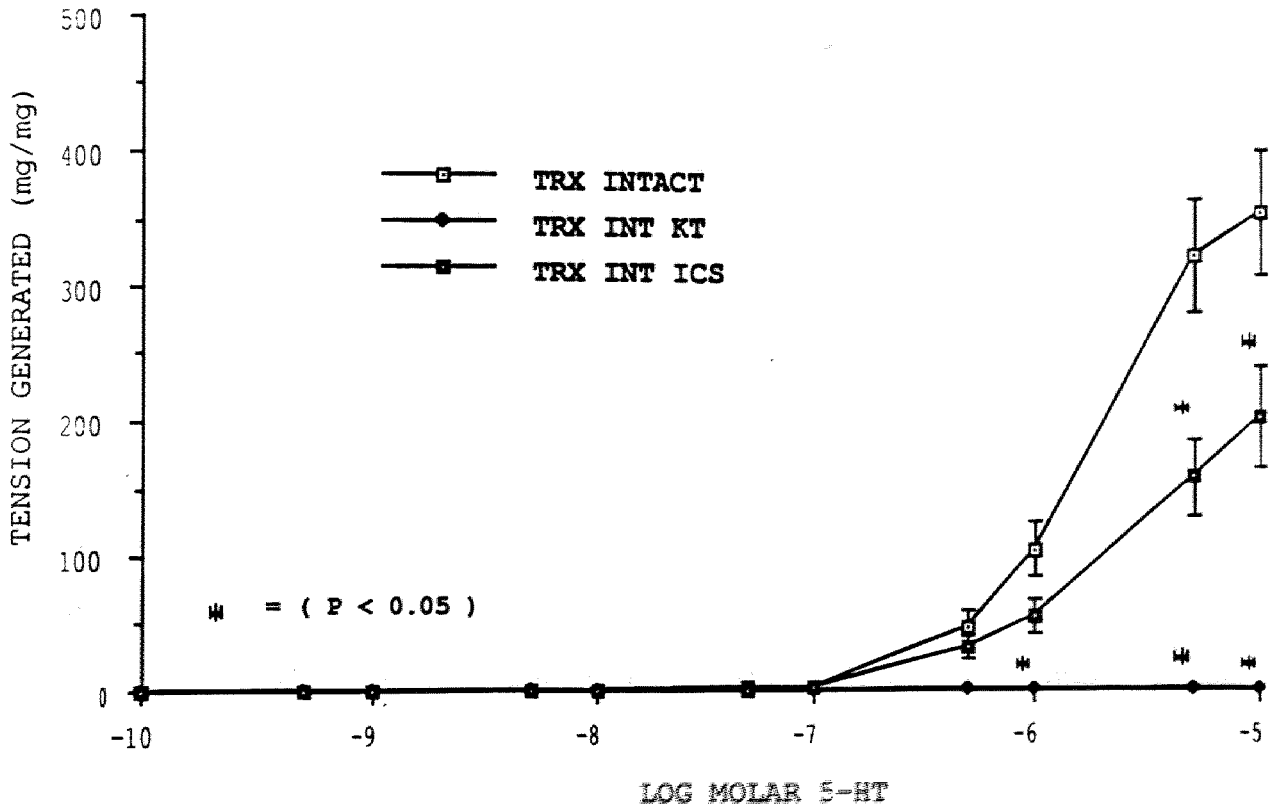


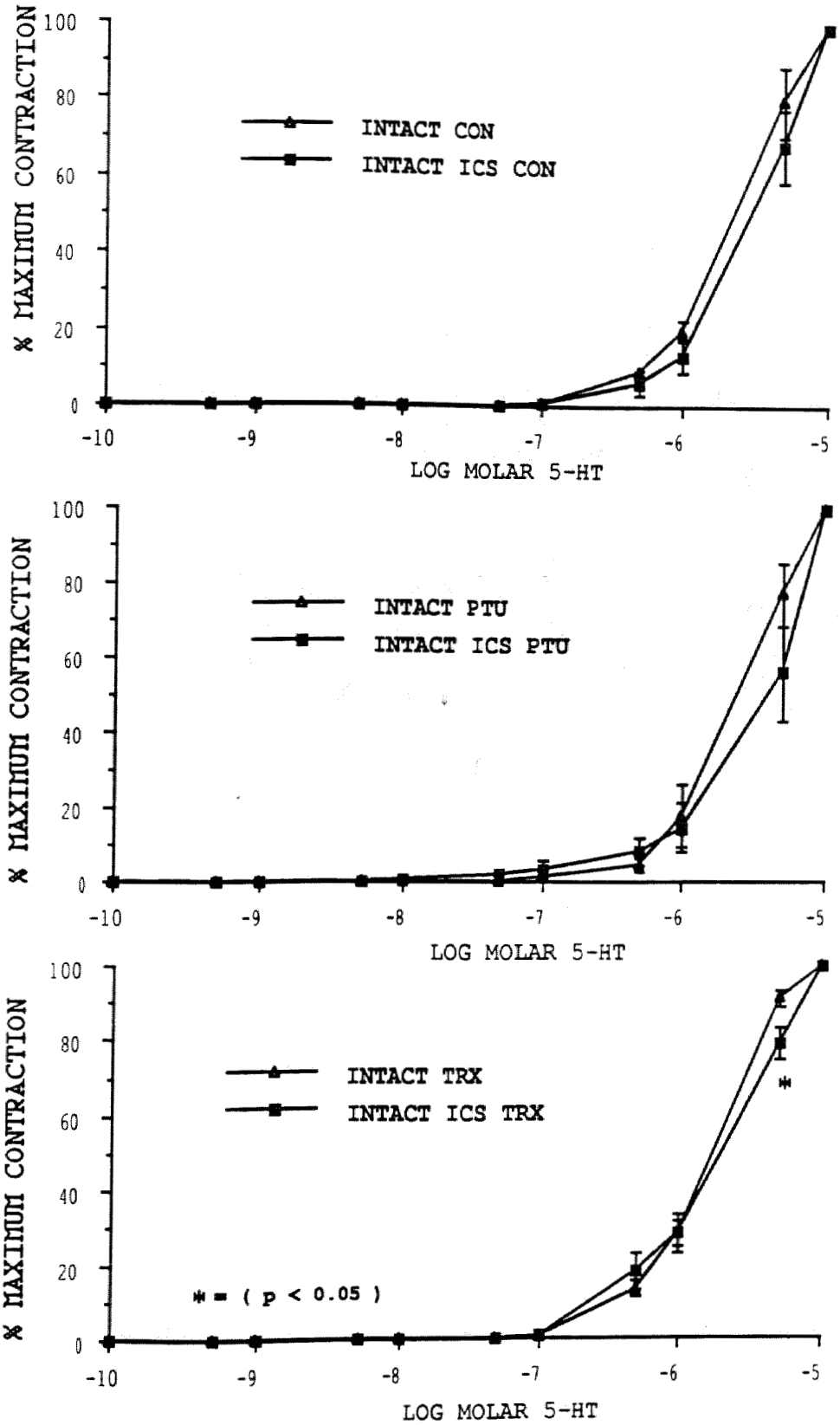
FIGURE 7c. Effects of cumulative doses of 5-HT on intact and ketanserin or ICS 205-930 pretreated aortic rings from hyperthyroid rats.

and 10 μM concentrations of 5-HT in light of the apparent lack of 5-HT_3 sites on rat aorta (Figure 7c).

Percent Maximum Response to 5-HT in Intact Rings Pretreated with ICS 205-930 and Ketanserin

In all thyroid states, the percent maximum response was not significant between the ICS 205-930-treated and - untreated rings (Figures 8a,8b,8c). Micromolar concentrations of the 5-HT_2 antagonist ketanserin effectively inhibited tension development in the three thyroid states (Figures 7a,7b,7c). The ketanserin-treated intact rings did not generate discriminable tension, and therefore calculation of the percent maximum contraction measure is inappropriate.

As another measure of the effects of 5-hydroxytryptamine on rat vascular smooth muscle, the effective dose needed to produce half-maximal contraction (ED_{50}) was compared in the thyroid states in the presence or absence of selective 5-HT receptor antagonists(Figure 9). The ED_{50} of the hyperthyroid denuded tissue was significantly lower than the denuded euthyroid and the intact hyperhyroid tissue.



FIGURES 8a,8b,8c. Percent maximum contractions of cumulative concentrations of 5-HT on intact or ICS 205-930 pretreated aortic rings from euthyroid, hypothyroid, and hyperthyroid rats.

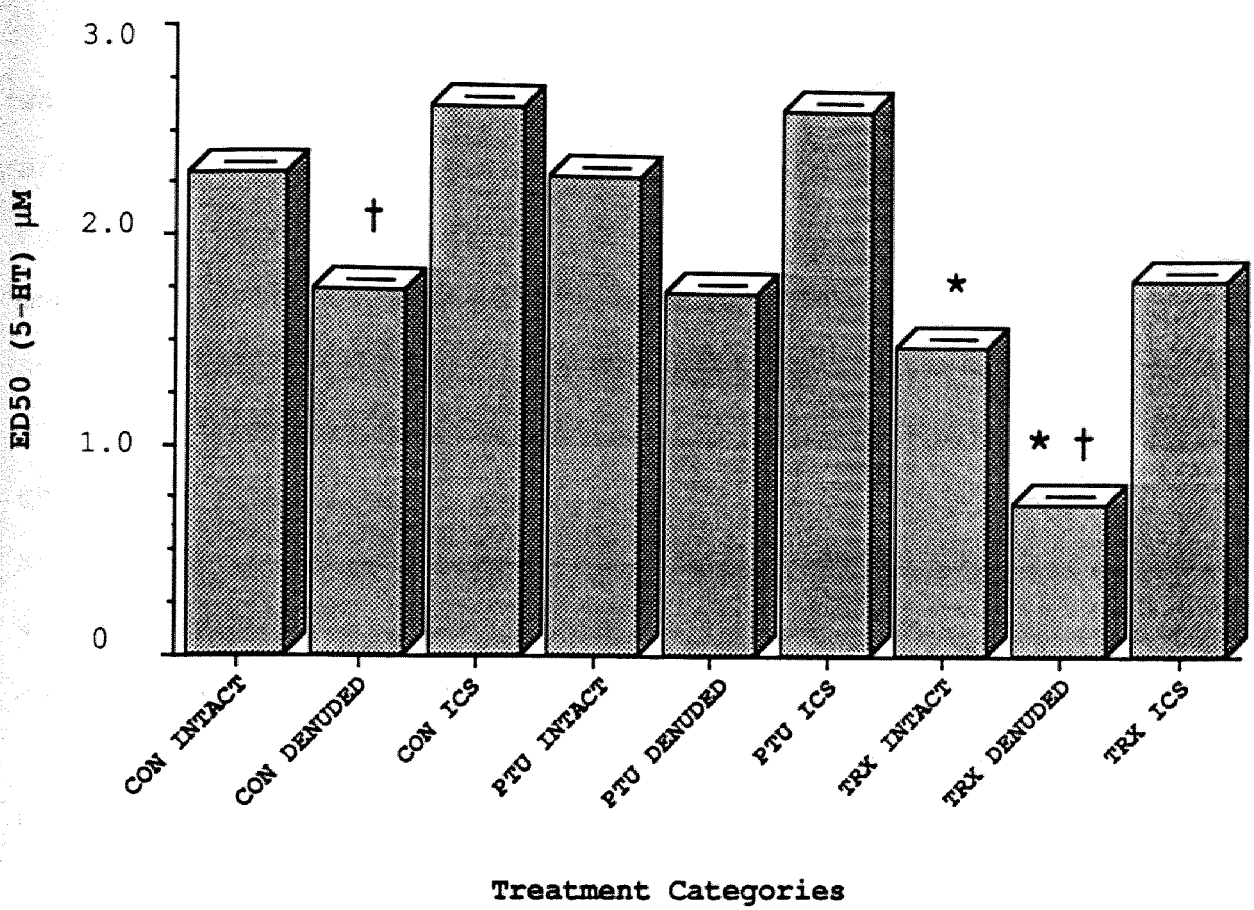


FIGURE 9. ED50 values of thyroid states and treatments in response to 5-HT. The symbols denote a significant difference between hyperthyroid denuded aortic rings and hyperthyroid intact rings(*) and between the hyperthyroid denuded rings and control denuded(†) rings (p=0.01). Standard error was calculated by one factor ANOVA.

DISCUSSION

The first parameter measured in the three thyroid states was the rate of repolarization of the vascular smooth muscle after a maximal contraction with 55mM KCl. The rate of repolarization is indicative of restoration of the resting membrane potential and cellular calcium efflux, and/or the sequestration of calcium into the sarcoplasmic reticulum. Endothelium-intact and denuded aortic rings in the three thyroid states showed a differential relaxation after a maximal contraction elicited by potassium chloride (Figures 4a-4e). Previous work examining the effects of thyroid state on KCl-induced contractions showed no significant differences in percent maximal response, while significant differences in tension generated were elicited at KCl concentrations higher than 45mM (Hade 1985). In response to KCl concentrations greater than 45mM, hypothyroid rings generated significantly more tension while the hyperthyroid rings generated significantly less tension than the control (Hade 1985). A comparable study of the responses of thyropathologic rat aorta to increasing doses of KCl found that at concentrations higher than 20mM, the dose-response curve of the hypothyroid tissue was shifted to the right as compared to the euthyroid tissue, while the hyperthyroid tissue demonstrated a significant inhibition (Gunasekera and Kuriyama 1990). The data shown in figure 4a are in agreement with those of Field et al.(1973) demonstrating decreased relaxation rates in

hyperthyroid and increased relaxation rates in hypothyroid rat aorta after a KCl-induced(80mM) contraction. In addition to significant differences between the three thyroid states in intact and denuded tissues, significant differences in the rates of relaxation between the intact and denuded thyroid states were also demonstrated (Figures 4c,4d,4e). These data suggest that endothelium-dependent mechanisms may be involved in the restoration of the resting membrane potential of the thoracic aorta. Ash et al.(1972) found reduced binding of calcium to skeletal sarcoplasmic reticulum fragments in thyroxine-treated cats. Thyroid hormones have also been shown to decrease the density of calcium channels in rat vascular tissue (Hawthorn et al. 1988). These findings suggest that hyperthyroid aortic tissues have a reduced capacity to sequester calcium into the endoplasmic reticulum, therefore this effect may subserve differences between calcium-dependent responses in thyropathological tissues. Chemically induced hyperthyroidism produces elevated amounts of the α-subunit of the G_i binding protein and the ß-subunit found in all G-binding proteins in adipocytes of rats (Milligan et al. 1987). The regulation of K^+ and Ca^{2+} channels by G-proteins has been documented in several reports (Brown and Birnbaumer 1988, Hofmann et al. 1987, Rosenthal and Schultz 1987). The repolarization rate may be faster in the hypothyroid aortic rings due to decreased degradation and/or increased synthesis of G-protein subunits which regulate K^+-Ca^{2+} channels.

An additional factor to consider when assessing the contractile properties of vascular tissues is the ability of the endothelial cells to modulate tension through the release of vasoactive substances. In response to vasotropic substances such as 5-HT, the endothelium may release EDRF, EDCF, and endothelin to exert effects on vascular smooth muscle cells. The endothelin receptor is coupled to phospholipase-C via a pertussis-insensitive G-protein (Takuwa et al. 1990). The main pool of calcium that is mobilized by endothelin is apparently derived from the activated Ca^{2+} influx through voltage-independent calcium channels (Huang, Hisayama, and Takayanagi 1990).

Changes in serotonergic receptor number within specific brain regions have been observed during phases of the rat estrous cycle (Biegon, Bercovitz, and Samuel 1980) and different thyroid states (Mason et al. 1987), demonstrate the plasticity of 5-HT receptors in response to the hormonal milieu. In the presence of high concentrations of thyroid hormone (500µg/kg), 5-HT_2 receptor binding (B_{max}) was increased in rat cerebral cortex and striatum (Mason et al. 1987). An increase in the B_{max} of 5-HT_2 receptor binding sites in response to thyroid hormone might reflect with an increase in the tension generated in the hyperthyroid rings (Figure 5a). Although direct comparisons between tension generated and B_{max} cannot be made, I posit that changes in tension generated may reflect changes in B_{max} and/or K_D. The results of Coville and Telford (1970) show a decrease in the

sensitivity of thyroxine-treated guinea-pig aorta. The use of a different experimental animal and tissue localization may account for these observed differences. The slight increase in tension observed in denuded tissues as compared to the intact tissues suggests that the endothelium releases a substance which induces relaxation in the vascular smooth muscle. EDRF may subserve this effect. The absence of EDRF in denuded preparations would thus reveal the full 5-HT-induced smooth muscle contraction. This effect was evident in the hypothyroid (PTU) tissues (Figure 5c). The increased contraction of the denuded hypothyroid rings suggests an enhancement of an endothelial-originating dilatory pathway and/or a increase in smooth muscle 5-HT-mediated response. The former may play a greater role of the overall tension generated, since a significant decrease was observed in the intact hypothyroid tissue when compared to the intact control tissue. Consequently, this significant decrease was abolished in the absence of the endothelium. Schoeffter and Hoyer (1990), speculated that the 5-HT receptor mediating endothelium-initiated relaxation in pig coronary arteries is of the 5-HT_{1D} subtype. It is known that the 5-HT_{1D} is negatively coupled to adenylate cyclase via the inhibitory guanine nucleotide binding protein(G_i)(Schoeffter et al. 1988). The α-subunit of G_i-protein has been shown to increase 5.6 fold in chemically induced hypothyroid adipocytes (Milligan et al. 1987). It is possible that the efficacy of 5-HT binding to the 5-HT_{1D} receptors is increased

in the hypothyroid state. Thus, the endothelium-intact hypothyroid tissue would have an enhanced relaxant response as compared to intact, control tissues. When the endothelium is removed in the hypothyroid tissue, this dilatory response is abolished, and a greater contractile response is observed.

The percent maximum contraction, which is a measure of tissue response-sensitivity, was significantly greater in the denuded hyperthyroid state as compared to the denuded control. The ED_{50} values were also significantly different between the denuded hyperthryoid and denuded control aortic rings. This increased contractile potency of 5-HT in the hyperthyroid tissue suggests that removal of the endothelium may reveal thyropathology-induced sensitization of a 5-HT receptor subserving contraction (i.e. 5-HT_2). Furthermore, as this sensitization is observed in the absence of the endothelium, it may be that the 5-HT receptor involved in this thyropathological effect is localized to the vascular smooth muscle. The ability of ketanserin to abrogate the response of the aortic tissue in the presence of high concentrations of 5-HT emphasizes the role of 5-HT_2 receptors in mediating vascular smooth muscle contraction. As well, the significant difference in sensitivity (percent maximum response and ED_{50} values) between intact and denuded hyperthyroid tissues strengthens the concept that an endothelium-derived substance may be inhibiting contraction (i.e. 5-HT-mediated release of EDRF). Taken together, these data suggest an increased sensitivity in thyroid hormone-

induced contributions from 5-HT-mediated dilatory responses on the endothelium, and contractile responses on the vascular smooth muscle. Since the observed changes are in sensitivity rather than tension generated, these differences may indicate alterations in the affinity, or increased amplification of second messengers subserving the contractile cascade. A conceivable component of this magnification may be mediated by an altered coupling potency of the heterotrimeric G-protein.

Additional evidence confirming the involvement of 5-HT receptors is derived from the differential abilities of the 5-HT_3 receptor antagonist ICS 205-930, and the 5-HT_2 receptor antagonist ketanserin to attenuate the 5-HT-induced response in rat vascular smooth muscle. The inability of ICS 205-930 to significantly reduce the response of the control and hypothyroid aortic rings, is in agreement with the reported absence of 5-HT_3 receptors in this tissue (Jazayeri et al.1989). It is tempting to speculate that the significant differences seen in ICS 205-930-pretreated hyperthyroid tissues at the highest doses of 5-HT may represent a component of the contractile response mediated by a unique 5-HT receptor. However, it is more likely that this effect reflects nonspecific binding of ICS 205-930 to other 5-HT or perhaps non-5-HT receptor sites. The nonsignificant differences in the sensitivities of the ICS 205-930-treated control and thyropathologic tissues may indicate the apparent lack of 5-HT_3 receptors and/or ligand specificity in these

tissues regardless of thyroid state. Further studies are required to more clearly define these possibilities.

CONCLUSIONS

The effects of thyropathology on vascular tissues are complex. This thesis investigated the response of rat aor vascular smooth muscle to 5-HT in altered thyroid states. Several mechanisms may be responsible for the thyropathol induced alterations of 5-HT-evoked contractile responses. These include putative alterations in the availability of endogenous 5-HT, the level and/or sensitivity of functiona 5-HT receptors, the composition of the signal transductior network, and the response capacity of the endpoint substra of signal transduction. All of the aforementioned factors may be involved to varying degrees in the differential contractile properties of the thyropathologic rat thoracic aorta. Specifically, this thesis supports previous resear that 5-HT mediates the release of a vasodilatory substance EDRF, from the endothelium. Furthermore, the increased contraction seen in denuded hypothyroid rings suggests the enhancement of an endothelial-originating dilatory pathway and/or a increase in the smooth muscle 5-HT-mediated response. The increased contractile potency of 5-HT in th hyperthyroid rings suggests that removal of the endotheliu may reveal thyropathology-induced sensitization of a 5-HT_2 receptor type localized to the vascular smooth muscle. A significant difference in sensitivity between the intact a denuded hyperthyroid tissue suggests the activity of an endothelium-derived substance which inhibits contraction

(i.e. 5-HT-mediated release of EDRF). The 5-HT_3 antagonist ICS 205-930 did not significantly effect the responses of the control and hypothyroid tissues. At the highest doses of 5-HT, ICS 205-930-pretreated hyperthyroid tissues generated significantly less tension than the intact hyperthyroid tissues. In the abscence of 5-HT_3 receptors in rat thoracic aorta, the attenuation by ICS 205-930 likely reflects nonspecific interactions of this ligand with other substrates, as demonstrated by the nonsignificant differences in the sensitivities of the ICS 205-930-treated control and thyropathologic tissues.

The characterization of 5-HT receptor responses on the vascular aortic smooth muscle and endothelial tissue in altered thyroid states is crucial to the understanding of the profound effects mediated by an excess and deficiency of thyroxine. The heterogeneity, location, and cellular responses evoked by the 5-HT receptor types in response to 5-HT in euthyroid and thyropathological vascular smooth muscle emphasize the importance of further studies of the pharmacological and physiological properties of this system.

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